GUIDELINES FOR pH ADJUSTMENT OF EFFLUENT SAMPLES FOR TOXICITY IDENTIFICATION AND REDUCTION EVALUATIONS

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GUIDELINES FOR pH ADJUSTMENT OF EFFLUENT SAMPLES FOR TOXICITY IDENTIFICATION AND REDUCTION EVALUATIONS

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ABSTRACT

This document provides guidelines for performing pH adjustments of samples of liquid effluents for acute lethality toxicity testing. It is intended to be used by dischargers of wastewater for an evaluation of the causes of toxicity as measured by protocols for laboratory testing of trout and of *Daphnia*. The data so generated may provide some information on the characteristics of the toxicants and on the remedial measures needed for compliance with limits for effluent toxicity.

Toxicity tests of pH adjusted effluent samples are conducted in addition to toxicity tests of unaltered samples. Chemical adjustment of pH is open for samples that are outside the pH range 5.5 to 9.5 and are significantly lethal to trout or *Daphnia*. The pH adjustment endpoint is 5.5 to 6.0 for "acidic" (pH < 5.5) and 9.0 to 9.5 for "alkaline" (pH > 9.5) wastewaters and is performed by adding acid or base to a sample of full strength (100%) effluent. An adjusted pH in the 6.0 to 9.0 range is an "overshoot" and would invalidate the sample for toxicity testing.

Although further decreases in the toxicity of some effluent samples would be expected with adjustments into the pH range 6.0 to 9.0, the objective is to adjust the pH to the outer limit of the nonlethal range for full strength effluent, while minimizing the increase in sample volume (effluent dilution) and in ionic strength (salt formation) due to the added amount of acid or base.

Besides causing direct toxicity, the pH of aqueous solutions has a profound influence on the physical-chemical and toxic properties of many organic and inorganic substances. Therefore, toxicity removal associated with an adjustment of pH is difficult to interpret. Further, more detailed tests of many samples are usually necessary to identify and confirm the causes of effluent toxicity.

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INTRODUCTION

Chemical adjustment of effluent pH can be an effective measure for water pollution control. For example, some mining industries neutralize the pH of acidic wastewater with lime or other bases to promote the breakdown of cyanide and the precipitation of trace metals. In this regard, a chemical adjustment of the pH of an effluent sample for toxicity testing can be a useful investigative procedure if it is part of a Toxicity Identification and Reduction Evaluation (TIRE). TIREs provide methods for characterizing and identifying the toxicants in complex mixtures and for determining the remedial measures needed for compliance with limits for effluent toxicity (see the Appendix). The guidelines here are intended to assist the experimental design of the first phase of a TIRE, in which pH adjustment is used as a method to prepare effluent samples for other treatments such as aeration, filtration and chromatographic separation. The following must be considered when undertaking pH adjustments and interpreting the results from toxicity tests:

Effluent toxicity studies are interdisciplinary and their success requires open communication between toxicologists, chemists and engineers. In order for the data to be useful to all concerned parties, the investigative team must customize an experimental design to answer well-defined questions about the toxicity and variability of an effluent.

Each effluent is unique in its composition and each sample of a particular effluent may be different than other samples in its physical-chemical and toxic properties. This is due, in part, to the variability over time in the presence and concentrations of different effluent constituents that can contribute to acute lethality. Therefore, it is essential to collect and test many samples to understand the variability of an effluent.

The requirements for quality assurance and quality control in analytical chemistry and aquatic toxicity studies are not always compatible, yet both must be addressed. Procedures to ensure the quality of toxicity data may impact on the results of chemical manipulations and vice versa. For example, sample aeration is a requirement of the trout test, yet this is likely to change the pH. When testing the toxicity of chemically altered samples, concessions must be made depending on the importance and use of the data.

Opportunities are limited to retest an effluent sample to confirm an initial result. The differences observed over time in the toxicity of a sample can be attributed to changes in its physical-chemical and toxic nature and to experimental error. Conversely, the toxicity of an effluent sample may be stable over time, but the causal agent(s) may still change with sample age. Therefore, controls and blanks must be included in the experimental design to make usable results from one set of tests and only fresh samples should be used.

Working with wastewater samples of unknown or ill-defined composition requires care and caution to avoid chemical exposure via inhalation and skin absorption when collecting, handling and manipulating samples. This is especially true when using strong solutions

of acid or base on large volume samples, as is discussed here. The user of this guideline is responsible for personal health and safety and should be thoroughly familiar with good laboratory practices before manipulating samples of effluent and testing them for toxicity.

Although toxicity tests of pH adjusted effluent samples can be useful for research purposes such as a TIRE, regulatory toxicity tests of whole effluent must be performed on intact samples that have not been altered chemically for pH. Environment Ontario conducts regulatory toxicity tests of wastewater because it has the mandate to assess and control the impacts of effluents on aquatic life (MOE, 1984). The toxicological uncertainties associated with the known limitations of the chemical-by-chemical approach are covered, in part, by the use of whole effluent toxicity tests as an integrated measure of the combined (matrix) effects of the effluent constituents.

An adjustment of pH is not permitted for regulatory toxicity testing because a modified sample of an effluent no longer simulates the actual discharge to the environment. Also, toxicity data from a pH adjusted sample of effluent are difficult to interpret outside of a TIRE because the adjustment is performed by adding strong acid or base to an effluent sample and this changes more than the pH. For example, acid or base additions can result in the formation of new toxic agents; release sorbed chemicals into solution by destroying, dissolving or altering particles to which the chemicals were bound; cause nonpolar organic solutes to sorb to uncharged surfaces because of the "salting out" effect of increased ionic strength.

EFFLUENT SAMPLING AND HANDLING

Samples of effluent are perishable and any preservation other than temperature control (5 to 15°C) would invalidate it for toxicity testing. Refrigeration and rapid shipping can maintain the integrity of most samples. Some samples are so perishable that on-site testing or special procedures may be necessary for their collection and handling. All samples taken for toxicity testing should be subsampled for a chemical analysis of the parameters that are considered relevant to its toxicity. If a toxic sample is identified, the data from a chemical characterization may help the search for toxicants.

When undertaking adjusted pH tests, enough volume must be available to set up a full dilution series of pH altered and unaltered sample. Therefore, sample volumes should be a minimum of 10 litres for *Daphnia* and 160 litres for trout tests. Additional treatments (for example, aeration, filtration and chromatographic separation) and testing of pH adjusted and unadjusted samples, as in a TIRE program, would require larger volumes of effluent. Sample containers should be lined with food-grade polyethylene or polypropylene bags or made of stainless steel, glass or perfluorocarbon. Any materials coming into contact with the sample must be inert, clean and nontoxic. The sample containers must be filled and sealed without head space. There should be no preservatives used in the sample and the sample should not be aerated during storage.

A written record is essential to identify the source of the sample and how it was handled. This record must contain: the industry/company or source name, its location, the name or numerical identifier of the specific location at the site where the sample was taken and the date and time of sampling. It should contain: the name of the sampler, the method of sampling (grab or composite) and the conditions of shipment (temperature of refrigeration). The sample label should comply with appropriate labelling requirements for shipping the materials involved.

If samples of effluent are required for "legal tests", a tape or label seal should be attached across the top edge of the sample container with the initials of the sampler and a serial number on it. The sampler should record the serial number when the seal is attached and the analyst should record it when the seal is broken at the testing laboratory. The objective is to be able to testify, with absolute certainty, that the sample was not tampered with because it was not left unattended when unsealed. This fulfils the legal requirement for "continuity of evidence".

As soon as possible after receipt by the toxicity testing laboratory, the temperature of the sample should be recorded. The sample must not be frozen, but should be chilled if possible. If more than 48 hours elapse before testing, the sample must be refrigerated to between 5 and 15°C. If samples are shipped in several containers they must be recombined and mixed thoroughly (made homogeneous) before use.

PROCEDURES FOR ADJUSTING pH

The adjustment of pH and toxicity tests should commence as soon as possible but must be within five days (120 hours) of sample collection. The sample is valid for nine days for trout and seven days for *Daphnia* including the respective exposure periods.

Chemical adjustment of pH is open for effluent samples that are outside the pH range 5.5 to 9.5 and are significantly lethal to trout and/or *Daphnia*, as measured by protocols for toxicity testing. The pH adjustment endpoint is to 5.5 to 6.0 for "acidic" (pH < 5.5) and 9.0 to 9.5 for "alkaline" (pH > 9.5) samples of effluent. An adjusted pH in the range 6.0 to 9.0 is an "overshoot" and would invalidate the sample for testing. The pH adjustment is done slowly by titration using 1.0, 0.1 and 0.01 normal solutions of reagent grade NaOH or HCl in high purity deionized water. Continuous monitoring of sample pH with a sensitive, calibrated, solid state pH meter is essential.

A minimum eighty (80) litre sample of effluent is required for pH adjustment and a trout test. The mixing for a titration is best performed by hand with a length of PVC pipe, a perfluorocarbon or stainless steel rod. Mechanical mixers may be used but violent mixing is not permissible. The maximum allowable mixing energy by any method would create a vortex no greater than one third of the depth of the sample. A minimum five (5) litre sample of effluent is required for pH adjustment and a *Daphnia* test. Use a burette to add

acid or base to the sample volume in a beaker or flask on a stir plate, and a perfluorocarbon stir bar to mix for equilibration. Toxicity tests of both trout and *Daphnia* would require a single pH adjustment of a eighty-five (85) litre sample as indicated for the trout test.

Effluent samples must be allowed to equilibrate after each incremental addition of acid or base. The time required will depend on the buffering capacity of the sample. The total time for pH adjustment should be between 30 and 60 minutes. As the pH endpoint is approached, use weaker solutions of acid or base to avoid an overshoot into the 6.0 to 9.0 pH range. The objective is to adjust the pH to the outer limit of the nonlethal range for full strength effluent, while minimizing the increase in sample volume (effluent dilution) and in ionic strength (salt formation) due to the added amount of acid or base. Titration time and the volume and concentration of added acid or base must be recorded.

After the pH adjustment is performed on a sample of full strength effluent, no further pH adjustments are permissible once the toxicity test has been started. If the pH adjustment is intended to prepare effluent samples for additional treatments (aeration, filtration and chromatographic separation) then further pH adjustments of full strength effluent may be needed to maintain a desired pH for the treatment. Continuous monitoring of pH is essential through all stages of sample manipulation and testing.

TOXICITY TESTS

To determine the effects of pH adjustment on effluent toxicity, the acute lethality of both the unaltered and pH altered effluent samples must be measured simultaneously, under identical exposure conditions, using the same culture stock of test organisms distributed randomly among all of the exposure chambers. The toxicity test procedures are given in "Protocol to determine the acute lethality of liquid effluents to fish" (Craig et al., 1983) and "*Daphnia magna* acute lethality toxicity test protocol" (Poirier et al., 1988). The measurement of standard water quality parameters (temperature, pH, dissolved oxygen and conductivity) must comply with the minimum requirements of the trout and *Daphnia* test protocols. Parameters of concern, such as pH, should be monitored more closely over time. Dilution water must have consistent and known characteristics with respect to the standard water quality parameters.

Significant differences exist among species in their sensitivity to toxicants and adaptability to test conditions (for example, the range of pH and salinity for survival during an acute exposure). Therefore, the choice of species and toxicity test endpoint can change the conclusion reached. Generally, the direct toxic effects of pH as a lethal factor become more severe and rapid with a deviation in either direction from neutrality (CCREM, 1987). The pH range 6.5 to 8.5 meets the PWQO requirement for indefinite survival of aquatic life (MOE, 1984). The pH range 5.5 to 9.5 is nominally accepted as acutely nonlethal to most fish and invertebrates. Invertebrates are normally more sensitive than fish to

changes in pH. The pH of water can alter the biological characteristics (for example, membrane permeability, blood chemistry) of aquatic organisms and thus influence the pharmacokinetic disposition (uptake, distribution, metabolism and elimination) and toxicity of any chemical.

The toxicity test is the only analytical tool that can be used reliably to detect and quantify toxicity. Until the cause(s) of toxicity is identified, chemical analysis can provide only indirect clues about the identity of the toxicant. Organisms have a limited number of ways in which they respond (commonality of output) though the toxic agents may be different (diversity of input). Since organisms would respond to any effluent constituent that is above a threshold detection level, biological effect *per se* may not show the nature or identity of the toxicant. Therefore, it is necessary to test for the spurious addition and removal of toxicity due to the particular methods of pH adjustment; so-called artifactual toxicity.

Potential causes of toxic artifacts include: biologically stressful ionic strength resulting from salt (NaCl) formation due to the addition of strong solutions of acid or base; sample dilution by the added volume of weak solutions of acid or base; heterogeneous sample due to poor mixing; degassing and/or oxidation of toxicants due to vigorous or extended mixing; toxic levels of silver from calomel electrodes on pH meters. Because the usefulness of these procedures relies on interpretation of toxicity test results, the degree of sample manipulation is limited and the use of controls and blanks is necessary.

A **toxicity control** is a test of the unaltered sample in a comparison with a test of the altered sample. The purpose is to find a relative difference between two toxic conditions. For example, if a species other than trout is tested, the increased ionic strength caused by a pH adjustment may be toxic. This problem can be avoided by using more dilute solutions of acid or base for the titration. Salt toxicity can be assessed by adding an appropriate amount of NaCl to an aliquot of the unaltered sample, for testing as a **salt control**. Alternately, if the original NaCl concentration in the sample is known, the final concentration of NaCl can be calculated from the added volume and strength of acid or base and the final solution volume. A comparison of this calculated concentration of NaCl to toxicity values (LC50s) in the literature can be used as a rough guide to judge the degree of salt toxicity.

If large volumes of acid or base are used, this would dilute the sample and reduce the toxicity. This problem can be avoided by using stronger solutions of acid or base. An equivalent volume of dilution water could be added to an aliquot of the unaltered sample for testing as a **dilution control**. Alternately, the extent of dilution due to the added volume of acid or base can be used to "correct" the nominal exposure concentration when calculating LC50s. If the mixing energy and time required for titration of a sample is suspected of degassing and/or oxidizing toxicants, an aliquot of unaltered sample can be mixed similarly without adding acid or base for testing as a **mixing control**.

A **toxicity blank** is a toxicity test of dilution water that has been subjected to the same treatment as a sample of effluent to determine if the treatment has inadvertently added toxicity. A blank is not very useful for identifying toxic artifacts in chemically altered samples of an effluent because toxicity is affected by the wastewater matrix. For example, a toxicity blank for sample ionic strength (adding the same amount of acid or base to dilution water) is not appropriate because of differences between the wastewater and the dilution water in their initial ionic strength, composition and buffering capacity. In contrast, toxicity blanks are essential when additional treatments (for example aeration, filtration and chromatographic separation) are performed on the pH adjusted and unadjusted samples.

THEORETICAL CONSIDERATIONS

The water quality parameter pH is a logarithmic scale measurement of hydrogen ion activity or concentration. A pH of 7 is neutral, by definition, because it is 1×10^{-7} mole of both H^+ and OH^- per litre. Because pH is a logarithmic quantity, a small change in pH can be a large change in concentration, when expressed in mole/L. A related factor is buffering capacity, the ability of water to resist a pH change. Acidity (primarily due to dissolved carbon dioxide and salts of strong acids and weak bases) refers to the ability to resist a pH change when a base is added. Acidity is measurable up to about pH 8.3. Alkalinity, measurable down to pH 4.5, is mainly due to carbonate-bicarbonate buffering and high alkalinity is associated with high levels of calcium and magnesium (hardness).

Solution pH has a modifying effect on the physical form or speciation of many organic and inorganic solutes. This influences the polarity, solubility, volatility and stability of substances dissolved in water and can be manifested as alterations in aquatic toxicity. Due to the influence of solution pH on the chemistry and toxicity of solutes, pH adjustment can be a useful diagnostic tool for toxicant characterization when it is used with other treatments such as aeration and filtration of effluent samples.

The effect of pH on the physical form of acids and bases is quantified by a substance's equilibrium dissociation constant, commonly expressed as a pKa value. By definition, when solution pH = substance pKa, equal amounts of a substance exist in the ionized (dissociated) and unionized (molecular) form. Table 1 generalizes the relationship between solution pH, substance pKa and the ionization of an acid and base:

For example, a solution pH one unit lower than the pKa of an acid (pH=pKa-1) results in 90% of the acid in the unionized form and the remaining 10% is in the ionized form. At a solution pH two units below the pKa, 99% is unionized and 1% is ionized. This rule of thumb is approximate and does not apply to substances that are significantly dissociated into more than two forms at a given solution pH (i.e., substances such as boric acid with two pKa's less than three units apart). The pKa describes an equilibrium condition and does not provide information on the rate of the pH-driven reaction.

Table 1.

water pH	acid %unionized	%ionized	base %unionized	%ionized
pH=pKa+2	1	99	99	1
pH=pKa+1	10	90	90	10
pH=pKa	50	50	50	50
pH=pKa-1	90	10	10	90
pH=pKa-2	99	1	1	99

The effect of pH on the concentrations of ionized and unionized forms has several impacts on the physical-chemical and toxic properties of acids and bases. Ionized forms are polar and therefore have a high affinity for polar solvents such as water. These forms have a high water solubility and are not readily removed from wastewater by aeration or sorption to suspended particles which may be present. Ionized forms in solution are also considered less bioavailable. They do not readily penetrate biological membranes that separate aquatic organisms from the ambient water. Due to their low bioavailability, ionized forms can exert a given toxic effect only at a relatively high water concentration.

In contrast, substances in the unionized or molecular form are nonpolar and therefore have a low affinity for water. Low solubility in water (hydrophobicity) "drives" molecular forms out of solution into other phases such as air, suspended particles and even aquatic biota. Therefore unionized forms are more amenable to removal from solution by air stripping, filtration of particulate matter and extraction with nonpolar solvents. Unionized forms in solution are also considered more bioavailable; they can partition into and across biological membranes and adopt a tissue concentration in biota that can be orders of magnitude higher than the water concentration exposure (bioconcentration). As a result, a toxic effect can be caused by a relatively low water concentration and aquatic toxicity is judged to be higher for solutes in the unionized form.

For example, a given water concentration of the resin acid dehydroabietic acid (pKa = 7.25) is most toxic in an acidic solution because a significant mole fraction is present in the unionized form (Taylor et al., 1988). If solution pH is increased, the percentage unionized is decreased and the aquatic toxicity will be less than observed at a lower pH for the same given concentration. Chlorophenols, ammonia and sulphide are other examples of commonly occurring pollutants that can cause acute lethality and that are subject to alterations in form and toxicity due to changes in pH. The chemistry, bioavailability and toxicity of inorganic metal-ion complexes (for example, manganese) also are influenced by pH conditions.

It is difficult to determine whether pH dependent differences in toxicity are due solely to a difference in bioavailability between chemical forms resulting in different amounts of

speciated chemical at the same site of toxic action. There also may be differences between forms in their efficacy and in their mode and site of toxic action. These points are discussed in more detail by Konemann and Musch (1981), Saarikoski and Viluksela (1981; 1982) and Saarikoski et al. (1986).

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APPENDIX

TOXICITY IDENTIFICATION AND REDUCTION EVALUATION

A Toxicity Identification and Reduction Evaluation (TIRE) provides investigative methods for determining the characteristics of toxicants in complex mixtures and the remedial measures for compliance with toxicity limits for wastewater discharges. Since each effluent is unique in its composition and variability relating to the specific operation of an industrial facility, it is not possible to provide one protocol for universal application. The following is intended to provide some general assistance and advice for the design and conduct of a toxicity evaluation. A more detailed description of TIREs and several case studies are given in Fava et al. (1989).

When an effluent is toxic repeatedly, the first step is to investigate the in-plant practices that may be the source of the toxicity problem. Some areas to be examined are facility "housekeeping" (for example, spill control), treatment plant optimization for removal of its design parameters and the selection and use of process and treatment chemicals. If "best management practices" cannot produce a nontoxic effluent, then a Toxicity Identification Evaluation (TIE) will be necessary.

TOXICITY IDENTIFICATION EVALUATION

A TIE is a systematic experimental approach to characterize, identify and confirm the physical-chemical nature and/or identity of the toxicant(s) in wastewater. Initially, a parallel series of benchtop treatments (for example, filtration, aeration, chromatographic separation) are used to alter, remove and fractionate the potential toxicants in an effluent sample. Simplified toxicity tests are conducted on these effluent fractions to determine which treatments reduced or removed the acute lethal effects that were observed for the intact sample. Such information can indicate that the toxicant is of a particular chemical class, for example, organic acids or cationic metals. This can be used to narrow down the search for a chemical-specific analytical procedure to uncover the identity and source of the toxicant. If the identity of the causative agent remains unknown, the TIE will have at least provided some information on its volatility, hydrophobicity, reactivity, etc. This may help to select a treatment technology that produces nonlethal effluents.

A single effluent treatment test (for example, parallel toxicity tests of a pH adjusted and unadjusted aliquot of a wastewater) does not, by itself, provide proof that a certain chemical or class of chemicals is causing toxicity. One must perform further, more detailed tests on many samples to provide the "weight of evidence" necessary to characterize, identify and confirm causal relationships between TIE tests and the toxicity of an effluent. The diagnostic value of a TIE can be limited if it does not include the full set of tests that are available (Mount and Anderson-Carnahan 1988a; 1988b; 1988c).

SOURCE IDENTIFICATION OR TREATABILITY STUDIES

Based on the results of a TIE, a discharger would then search the industrial facility (using chemical-specific analysis) for the upstream origin of the toxicant and control it at source. If the identity of the toxicant is known, source identification and control may be more efficient because it is usually easier to deal with smaller, concentrated side streams than with large dilute final effluents. However, the search for toxicant source streams becomes more difficult in complex facilities with variable production schedules and processes. It is also more difficult if the TIE has revealed a physical-chemical class of toxicants in the final effluent rather than a specific chemical. If this approach is not practical then an evaluation of treatment methods for removal of the toxicant from the final effluent is warranted. A treatability study of the final effluent can normally result in resolution of a toxicity problem. This usually would require the modification or construction and maintenance (by qualified personnel) of an "ex-plant" facility for treatment of wastewater.

The two approaches (source identification and treatability) are not mutually exclusive and some combination may be needed. The design of a facility-specific study would consider the results of the TIE, the ease of treating the final effluent, the number of possible source streams, the ability to modify facility operations and the variability of the toxicants.

TOXICITY REDUCTION METHODS

Remedial options for toxicity reduction include source reductions, modifications in waste treatment operations and additional treatment technologies. Source reductions are the practices and procedures to reduce or eliminate chemical loads from the most upstream end of a process to the point of influent to the treatment plant. It may involve the selection and use of process and treatment chemicals (for example, chlorine substitution), process modifications, waste stream batching or segregation, pretreatment of source waste streams, materials recovery or waste recycling.

The activated sludge process although designed and operated to remove BOD, also can remove many metals and nonpolar organics as a side benefit. But the modification of waste treatment processes beyond optimization for the removal of its design parameters may still be required. Some areas to be examined include hydraulic and mass loading of the facility, chemical feed rates, biological enhancement, source stream batching or segregation, effluent "polishing" and additional treatment technologies.

Additional treatment technologies are often based on the rate and extent of physical-chemical processes. These technologies can result in the removal of many chemicals from wastewater simply because the chemicals share properties that make the treatment effective. For example, aeration removes volatile chemicals from solution; settling of solids removes hydrophobic (sorbed) chemicals from wastewaters that have a high concentration of particulates; adjustment of pH alters the water solubility of acids and

bases and can be used to maximize the effectiveness of other treatments such as aeration and solids settling. If the physical-chemical nature of the toxicants has been characterized, it is not always necessary to know their chemical identity to pursue a treatability approach. If more than one method is identified to reduce toxicity, then selection of the "best" method will be a facility-specific decision that considers cost, performance and flexibility. After the control method has been implemented, the final step is to continue biological monitoring to confirm that the toxicity limit has been attained and maintained.

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